A Toxicity Assessment Approach to Evaluating In-situ Bioremediation of PAH Contaminated Sediments

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ABSTRACT

Freshwater and marine sediment toxicity tests were used to measure baseline toxicity of sediment samples collected from New Jersey/New York Harbor (NJ/NY) (non-PAH-contaminated) and East River (PAH-contaminated) sediment (ERC). Four freshwater toxicity tests were used: (1) amphipod (*Hyalella azteca*) mortality and growth tests (a standard 10-day U.S. EPA method and two 7-day exposure methods [one using the standard amount of sediment, 100 ml; one using a reduced sediment volume, 17 ml] - the reduced volume freshwater amphipod test was developed and used in this study since existing volume requirements of the U.S. EPA standard method exceeded the amounts available from enhanced or natural attenuation treatment); (2) a 7-day aquatic worm (*Lumbriculus variegatus*) mortality and budding test; (3) a 7/8-day fathead minnow (*Pimephales promelas*) embryo-larval survival and teratogenic test (FHM-EL); and (4) a 4-day vascular aquatic plant (*Lemna minor*) frond number/growth/chlorophyll a test (Duckweed). Two marine tests were also used: (1) an amphipod (*Ampelisca abdita*) 10-day mortality test and a sheepshead minnow (*Cyprinodon variegatus*) embryo-larval sediment test (SHM-EL). ERC sediments were found to be highly toxic to all freshwater and marine organisms tested while the NJ/NY, non-PAH contaminated, sample showed no significant toxicity to the marine amphipod, but was slightly toxic to the freshwater worm and to freshwater and marine fish. For all tests run with freshwater organisms and the one marine amphipod no survival was found in any of the tests except for one of the freshwater amphipod tests (55%). The ERC sediment significantly reduced frond production (-58.3%) and chlorophyll a levels (-35.4%) in the freshwater duckweed test. To determine the cause of toxicity in the sediments, five sediment manipulations were performed: (1) a sediment purge procedure, where 2 to 4 volumes of lab water were replaced over the sediment in a 24-h period; (2) a sediment dilution procedure where, grade 40 silica sand was mixed with PAH-contaminated sediments on a weight:weight basis; (3) a sediment aeration procedure, where sediments were aerated by adding 80 ml of sediment (140 gms) to a 250 ml glass graduated cylinder and 120 ml of overlying water followed by aeration for 24-48 h; 4) an Ambersorb Treatment Procedure, where PAH-contaminated sediment samples were treated with two types of organic removal resins - Ambersorb 563 (AS 563) and Ambersorb 572 (AS 572); and (5) an Amberlite Treatment Procedure where IRC-718, an inorganic removal resin, was mixed with PAH-contaminated sediments. Results showed that freshwater amphipod survival was improved with the sediment aeration procedure and with 8% AS 563 and AS 572 treatments. Toxicity can also be reduced with the sediment dilution technique (100-fold). These manipulations revealed that hydrogen sulfide, organic compounds and inorganic compounds (metals) were factors in ERC sediment toxicity. Results from *Hyalella azteca* toxicity tests using ER and NJ/NY harbor sediment samples treated by an Aerobic Biodegradation Slurry System (BioSlurry) showed reductions in toxicity to the *H. azteca* equal to or greater than that achieved through chemical or mechanical manipulations of the samples. *H. azteca* survival in the various BioSlurry treatments of the ER sediment ranged from 35% to 65%, compared to survival of 20% in ER sediment treated by aeration and addition of 8% AS 572.

INTRODUCTION

Freshwater and marine sediment toxicity tests were used to measure baseline toxicity of sediment samples collected from New Jersey/New York Harbor (NJ/NY) (non-PAH-contaminated) and East River (PAH-contaminated) sediment (ERC). Four freshwater toxicity tests were used: (1) amphipod (*Hyalella azteca*) mortality and growth tests (a standard 10-day U.S. EPA method and two 7-day exposure methods [one using the standard amount of sediment, 100 ml; one using a reduced sediment volume, 17 ml] - the reduced volume freshwater amphipod test was developed and used in this study since existing volume requirements of the U.S. EPA standard method exceeded the amounts available from enhanced or natural attenuation treatment); (2) a 7-day aquatic worm (*Lumbriculus variegatus*) mortality and budding test; (3) a 7/8-day fathead minnow (*Pimephales promelas*) embryo-larval survival and teratogenic test (FHM-EL); and (4) a 4-day vascular aquatic plant (*Lemna minor*) frond number/growth/chlorophyll a test (Duckweed). Two marine tests were also used: (1) an amphipod (*Ampelisca abdita*)10-day mortality test and a sheepshead minnow (*Cyprinodon variegatus*) embryo-larval sediment test (SHM-EL).

PURPOSE: SEDIMENT TOXICITY TESTING

- To Determine how effective are developed Biotreatment Strategies in reducing ecotoxicity in contaminated sediments.
- To provide a measure of Biotreatment efficiency based on ecotoxicity values and to relate the reduction of contaminant concentration in sediments to the reduction of ecotoxicity based on biological assay methods.
- These toxicity methods are being used to assess how much each
- Biotreatment reduces lethal, sublethal or bioaccumulative levels of contaminants in sediments.

MATERIALS AND METHODS

Freshwater and marine sediment toxicity tests were used to measure baseline toxicity of sediment samples collected from New Jersey/New York Harbor (NJ/NY) (non-PAH-contaminated) and East River (PAH-contaminated) sediment (ERC).

- Four freshwater toxicity tests were used:
- Amphipod (*Hyalella azteca*) mortality and growth tests (a standard 10-day U.S. EPA method and two 7-day exposure methods [one using the standard amount of sediment, 100 ml;
- One using a reduced sediment volume, 17 ml - the reduced volume freshwater amphipod test was developed and used in this study since existing volume requirements of the U.S. EPA standard method exceeded the amounts available from enhanced or natural attenuation treatment)
- A 7-day aquatic worm (*Lumbriculus variegatus*) mortality and budding test;
- A 7/8-day fathead minnow (*Pimephales promelas*) embryo-larval survival and teratogenic test (FHM-EL); and a 4-day vascular aquatic plant (*Lemna minor*) frond number/growth/chlorophyll a test (Duckweed).
- Two marine tests were also used:
- An amphipod (*Ampelisca abdita*)10-day mortality test and a sheepshead minnow (*Cyprinodon variegatus*) embryo-larval sediment test (SHM-EL).

Differences in Standard and Miniaturized Procedures for *Hyalella azteca*, freshwater amphipod.

TEST CRITERIA	SPECIFICATIONS Standard Test	Miniaturized Test
Test Temperature	23°C ± 1°C	25°C ± 1°C
Test Chamber Size	300 ml	60 ml
Sediment Volume	100 ml	17 ml
Overlying Water Volume	175 ml	30 ml
# of Organisms/Chamber	10	10
# of Replicates	8	4
Test Duration	10 days	7 days

Procedures for Aquatic Worms, *Lumbriculus variegatus* sediment toxicity tests samples.

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	7 days
Temperature	25°C ± 1°C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	200 ml
Sediment Volume	40 ml
Overlying Water Volume	160 ml
Renewal of Test solution	daily
Age of Test Organisms	adults
# Organisms/chamber	10 or 20
# Replicate /Conc.	4
Organisms/Conc.	40 or 80
Feeding	1.5 ml or 2.0 ml FFAY*
Overlying Water	Reformulated Moderately Hard Reconstituted Water**
Control Sediment	grade 40 silica sand
Endpoint	Mortality and/or Growth
Test Acceptability	> 80% survival in controls

* = digested Fish Flakes/Alfalfa/Yeast
** = Hardness = 80 - 100 mg/L Alkalinity = 60 - 80 mg/L

Differences in Standard and Miniaturized Procedures for Freshwater Fathead Minnows *Pimephales promelas* embryo larval (FHM-ELS) and Marine Sheepshead Minnows *Cyprinodon variegatus* embryo larval (SHM-ELS) Sediment Toxicity tests.

TEST CRITERIA	SPECIFICATIONS Standard Test	Miniaturized Test
Test Chamber Size	125 m	60 ml
Sediment Volume	40 ml	17 ml
Overlying Water Volume	60 ml	30 ml

Procedures for Duckweed *Lemna minor* Sediment Toxicity Tests

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	4 days
Temperature	25°C ± 1°C
Photoperiod	14 hr light/10 hr dark
Test Chamber Size	30 ml
Sediment Volume	15 ml
Overlying Water Volume	2 ml
Renewal of Test solution	at 48 hours
Age of Plants	2 frond plants
# 2 frond Plants/ chamber	6
# Replicate Chambers/Conc.	4
# Plants/Conc.	48
Feeding	0.1 ml of 3 nutrient stocks
Overlying Water	Moderately Hard Reconstituted Water*
Control Sediment	grade 40 silica sand + alfalfa
Endpoint	Frond number Growth as wet wt. Chlorophyll-a
Test Acceptability	Number of control fronds doubles

* = Hardness = 80 - 100 mg/L Alkalinity = 60 - 80 mg/L

Sediment Manipulation Methods

To determine the cause of toxicity in the sediments, five sediment manipulations were performed:

Purge Procedure

- Purging of the sediment consisted of two methods 1) replacing overlying water in the first 24-hrs with 4-6 volumes and 2) a thin-layer purging method, where1.5L of sediment is placed in a shallow pan, with 15L of overlying water added. The overlying water was changed every 24H for 5 days and a sediment sample collected for pore water Unionized ammonia analysis. The pore water was collected by centrifuging the sediment sample at 2000 rpm for 20 minutes. Unionized ammonia was measured each day.

Sediment Dilution Procedure

- Grade 40 silica sand (wetted by mixing 100 ml of sand with 50 ml overlying water) was used as a dilution substrate. Sediment dilutions were made on a weight:weight basis. For example, 1% East River sediment was prepared by weighing out 1 gram of sediment, then 99 grams of control sand and mixing. A spoon or spatula was used to completely mix the materials and add them to the appropriate test container after which the overlying water was added. The diluted sediments were then treated as a standard sediment sample.

Sediment Aeration Procedure

- An aeration procedure was developed, to "blow-off" the volatile sulfides and oxidize the remaining sulfides, thus reducing the overall toxicity of these samples. The sediment samples were aerated by adding 80 ml of sediment (140 gms) to a 250 ml glass graduated cylinder and then adding 120 ml of overlying water. An airtube was then inserted into this mix and a mild aeration started (~100 bubbles/min). The cylinders were placed into the hood, covered and allowed to aerate overnight. After aeration, the slurry was removed from the cylinder, placed into centrifuge tubes and centrifuged at 2000 rpm for 20 minutes. After centrifuging, the excess overlying water was discarded and the sediment samples collected for use in the sediment toxicity tests. These aerated sediments were used as the 100% samples or diluted with sand as described above. If desired, the overlying water samples can be saved for aqueous testing with any species.

Ambersorb/Amberlite Treatment Procedure

- East River sediment samples were treated with two types of organic removal resins, Ambersorb 563 (AS 563) and Ambersorb 572 (AS 572). The resins were mixed in the sediments at a rate of 4% or 8% AS 563 or AS 572 by weight. Each sample was treated as described above in the procedure for sediment aeration.
- Amberlite IRC-718 is an inorganic removal resin. Data from others researchers indicate that the use of this type of resin can potentially reduce the toxicity associated with a sediment sample. IRC-718 procedure was the same as the Ambersorb except we used 8% by weight IRC-718 only.

Aerobic Biodegradation Slurry Systems

- Ten g contaminated sediment and 50 ml of natural overlaying water mixed on a rotary shaker in 160 ml glass serum bottles. The system is buffered by the addition of crushed limestone to the liquid.
- The headspace in the bottles contains pure oxygen to provide adequate oxygen (DO) in the liquid phase for the duration of the study.
- In a control set of serum bottles, a solution of sodium azide and sodium molybdate is added to prevent growth.
- Samples are analyzed for 19 contaminant PAHs, sulfate, pH and DO at the following time periods: 0,1,2,4,6, 8,12 and 16 weeks. The samples analyzed in the H. azteca toxicity tests were collected at the end of the 16 week treatment cycle.
- Salicylic acid (bioinducer), Ethanol and the surfactant Triton100 were evaluated as possible stimulants of the PAH biodegradative activity by the indigenous microbiota in the sediments.

RESULTS

Table 1. Survival data results for *Hyalella azteca* (Freshwater Amphipods), *Lumbriculus variegatus* (Freshwater Worm), Fathead Minnow Embryo larval survival (FHS ELS) *Lemna minor* (Duckweed) and *Apelisca abdita* (Marine Amphipod)

	H. azteca	*L. variegatus*	FHM ELS	SHM ELS	*L. minor*	*A. abdita*
Sample	% Survival	% Survival	% Survival	% Survival	% reduction Frond# / % reduction Chla mg/g	% Survival
NY/NJ Cnt	50 - 100	5 - 100	0	0 - 0	# -96% Chla -41%	95
1% NY/NJ	0-100		80	95	#0 Chla 0	
ERC	0- 55	0 - 0	0-0	0 - 0	# -94 to -58% Chla -25% to -35.4%	0
1 % ERC	0 - 73		0 - 10	20 - 80	#0 Chla 0	

NY/NJ Cnt = New Jersey/ New York Harbor PAH Uncontaminated Sediments
ERC = Eas[illegible] = Chlor[illegible]y weight of duckweed

Table 2. Comparison tests with *H. azteca* in both control and natural sediments using the reduced volume method.

	Reduced Volume Method				O[illegible] EMAP Method)			
Sample	Sur %	C.V. %	Grw [illegible]	C.V. %	Sur. %	[illegible] %	[illegible]w µg/sur	C.V. %
Cnt	100	0	[illegible]	12.3	100	0	77	25.8
ERC.	0	0	0	0	0	0	0	0

Cnt is the sand control sediment, spiked with 0.5% alfalfa.
ERC is the East River contaminated sediment.

Table 3. Results from *Hyalella azteca* sediment toxicity tests with East River contaminated sediment. The ER sediment was diluted to 0.1%, 1%, 10% and 100% for all treatments. The treatments included aeration, and aeration with the subsequent addition of 8% Ambersorb 572.

Sample	Sample Aerated	*H. azteca* % Sur	C.V. %
Sand Control	y	100	0
0.1% ER	y	100	0
1% ER	y	0	0
10% ER	y	0	0
100% ER	y	0	0
0.1% ER + 8% AS 572	y	95	10.5
1% ER + 8% AS 572	y	40%	40.8
10% ER + 8% AS 572	y	0	0
100% ER + 8% AS 572	y	20	141.4

Table 4. Results from *Hyalella azteca* sediment toxicity tests with East River contaminated sediment. The ER sediment was diluted to 1%, 5%, and 10% for all treatments.

Sample	Sample Aerated	*H. azteca* % Sur	C.V. %
Sand Control	y	100	0
1% ER	y	25	40
5% ER	y	25	100.7
10% ER	y	35	71.9
Sand Control	y	100	0
1% ER + 8% AS 572	y	90	12.8
5% ER + 8% AS 572	y	25	100.7
10% ER + 8% AS 572	y	65	64.4
Sand Control + 8% IRC 718	y	95	10.5
1% ER + 8% AS 572 + 8% IRC 718	y	45	42.6
5% ER + 8% AS 572 + 8% IRC 718	y	40	57.7
10% ER + 8% AS 572 + 8% IRC 718	y	30	115.5

Table 5. Results of *Hyalella azteca* sediment toxicity tests with East River contaminated sediment. The treatments included aeration, aeration with subsequent addition of 8% Ambersorb 563 and 572 and addition of 8% Ambersorb 563 with no aeration and 8% Amberlite IRC 718.

Sample	Sample Aerated	*H. azteca* % Sur	C.V. %
Sand Cnt	Y	100	0
10% ER	Y	0	0
10% ER 8% AS 563	N	0	0
10% ER 8% AS 563	Y	90	12.8
10% ER 8% AS 572	Y	85	11.8
10% ER + 8% AS 572 + 8% IRC 718	Y	30	115.5

Table 6. Results from two purging techniques 1) Replacing overlying water in the first 24-hrs. with 4-6 volumes until conductivity was reduced; and 2) The sediment was first treated using a thin layer purging method developed by USEPA Region II. In this method a 1.5L of sediment was spread over the bottom of a shallow pan and covered with 15L of test control water. The water was changed daily and the pore water total ammonia nitrogen analyzed. Once the tan dropped below 10 Mg/L the rest of the treatment procedures were performed. The ER sediment was diluted to 1% (using grade 40 Silica sand).

Sample Purged 1 or 2 Procedure	Sample Aerated	H. azteca % Sur	C.V. %
Sand Control 6/00	y-24H	100	0
1% ER P1 6/00	y-24H	0	0
1% ER P1 7/00	y-24H	25	40
1% ER P1 9/00	y-24H	45	22.2
1% ER P1 9/00	y-48 H	25	76.6
Sand Control 4/01	y-24H	100	0
1% ER P2 4/01	y-24H	35	54.7
1% ER P2 4/01	n	20	141.4

Table 7. Results from *H. azteca* sediment toxicity tests conducted using East River (ER) and New York/New Jersey Harbor (NY/NJ) sediments treated using an aerobic biodegradation slurry system (bioslurry) and the addition of materials to stimulate the PAH biodegradation activity of the indigenous microbiota in the sediments.

Sample	Treatment	H. azteca %Survival	C.V. %
Sand Cnt	none	90	22
NY/NJ	BioSlurry	65	15.4
NY/NJ	BioSlurry + Ethanol	90	12.8
NY/NJ	BioSlurry + Salicylic Acid	90	12.8
NY/NJ	BioSlurry + Triton	90	12.8
ER 1%	BioSlurry	80	20.4
ER 10%	BioSlurry	85	22.5
ER 100%	BioSlurry	40	40.8
ER 1%	BioSlurry + Ethanol	65	29.5
ER 10%	BioSlurry + Ethanol	75	33.6
ER 100%	BioSlurry + Ethanol	60	47.1
ER 1%	BioSlurry + Salicylic Acid	85	22.5
ER 10%	BioSlurry + Salicylic Acid	60	47.1
ER 100%	BioSlurry + Salicylic Acid	65	52.5
ER 1%	BioSlurry + Triton	85	22.5
ER 10%	BioSlurry + Triton	50	51.6
ER 100%	BioSlurry + Triton	35	97.6

Table 8. Analysis of ER sediments for selected organic and inorganic analytes. This includes the samples, the analytes of interest and the percent change over the period of treatment in the bioslurry system. Also included are the survival data in 100% samples from the tests conducted with the treated sediment samples.

	[illegible]	S[illegible]	[illegible]	[illegible]
	% [illegible]	% Chan[illegible]	[illegible]	[illegible]
naphthalene	-16.1[illegible]	-21.[illegible]	[illegible]	[illegible]
[illegible]alene	2.35	1.01	[illegible]	-27.34
[illegible]	-2.96	-3.76	[illegible]	21.60
[illegible]	-77.62	-77.52	[illegible]	-67.59
dibenzofuran	-58.45	-64.24	-51.18	-62.34
fluorene	-82.00	-81.49	-78.17	-65.84
phenanthrene	-72.85	-72.94	-73.26	-69.61
[illegible]	-81.52	-82.00	[illegible]	[illegible]
[illegible]	-66.47	-66.85	[illegible]	[illegible]
[illegible]	-67.42	-67.12	[illegible]	[illegible]
[illegible]	-60.53	-60.25	[illegible]	-60.06
[illegible]	-48.00	-46.22	[illegible]	-51.39
[illegible]	-25.80	-24.25	[illegible]	-23.57
[illegible]	[illegible]57	-5.82	[illegible]	-4.14
[illegible]	[illegible]37	-28.96	[illegible]	-18.05
[illegible]pyrene	[illegible]56	-21.00	[illegible]	-21.97
[illegible]nracene	41.03	33.89	[illegible]	[illegible]
[illegible]lene	8.16	-4.43	[illegible]	[illegible]
[illegible]	125.69	128.50	1[illegible]	[illegible]
Survival Toxicity	35.00	65.00	60.00	35.00

FIGURE 1

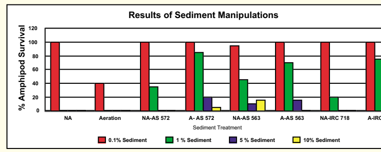


FIGURE 2

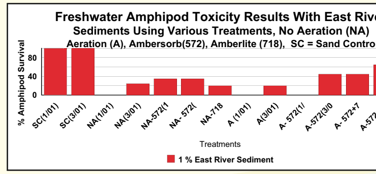
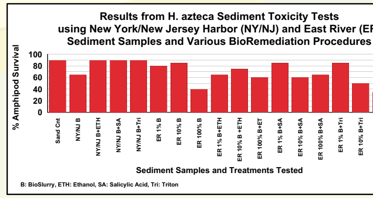


FIGURE 3



CONCLUSIONS

ERC sediments were found to be highly toxic to all freshwater and marine organisms tested while the NJ/NY, non-PAH contaminated, sample showed no significant toxicity to the marine amphipod, but was slightly toxic to the freshwater worm and to freshwater and marine fish. For all tests run with freshwater organisms and the one marine amphipod no survival was found in any of the tests except for one of the freshwater amphipod tests (55%). The ERC sediment significantly reduced frond production (-58.3%) and chlorophyll a levels (-35.4%) in the freshwater duckweed test. (TABLE 1)

2. The miniaturized method shows results similar to the old method for lethality. Modifications to feeding regime have improved the growth endpoints. (TABLE 2)

3. The miniaturized method will be very logistically desirable for assessing the success of treatment because 55% to 83 % less (17 ml vs 40 ml or 100 ml) sediment will be needed for testing.

4. Results showed that freshwater amphipod survival was improved with the sediment aeration procedure and with 8% AS 563, AS 572 and IRC 718 treatments. Toxicity can also be reduced with the sediment dilution technique (1000-fold). These manipulations revealed that hydrogen sulfide, organic compounds and inorganic compounds (metals) were factors in ERC sediment toxicity.(TABLES 3-6 and FIGURES 1&2)

5. Results from the tests conducted using samples from the Aerobic Biodegradation Slurry System (TABLE 7 and FIGURE 3) indicate the results from the tests with these sediment samples produced increases in *H. azteca* survival similar to those generated through earlier manipulations of the sediment using dilution, aeration and treatment of the sediment samples with organic and inorganic removal resins. Comparison of these results show the BioSlurry System does breakdown toxic components similar to those removed by the mechanical and chemical manipulations of the samples. Chemical analysis of the East River BioSlurry samples (TABLE 8) indicates significant reductions in the levels of a number of the organic analytes of concern in each of the different treatments.

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